Combination of heparin and aspirin is superior to aspirin alone in enhancing live births in patients with recurrent pregnancy loss and positive anti-phospholipid antibodies: a meta-analysis of randomized controlled trials and meta-regression

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Abstract

Objective. The combination of heparin and aspirin was regarded as the ‘standard therapy’ for patients with recurrent pregnancy loss (RPL) and positive aPL antibodies to enhance live births, but it largely stems from expert opinion. We performed a meta-analysis of randomized controlled trials (RCTs) to assess whether this combination works better than aspirin alone.

Methods. RCTs testing the efficacy of a combination of heparin and aspirin vs aspirin alone in patients with RPL and positive aPL antibodies were identified in electronic databases. Random effect meta-analysis was employed to pool relative risks (RRs) (with 95\% CI) of live births as the primary outcome. RRs of obstetrical complications and standardized mean difference of birth weight were the secondary outcomes. Mixed-effects meta-regression was performed to identify factors associated with live births.

Results. Data from five trials involving 334 patients were analysed. The overall live birth rates were 74.27 and 55.83\% in the combination and aspirin alone groups, respectively. Patients who received heparin and aspirin had significantly higher live birth rate (RR 1.301; 95\% CI 1.040, 1.629) than aspirin alone, with the number needed to achieve one live birth being 5.6. No significant differences in pre-eclampsia, preterm labour and birth weight were found between both the groups. Meta-regression using age at randomization, previous history of live births and episodes of miscarriages as covariates failed to predict the RR of live birth.

Conclusions. The combination of heparin and aspirin is superior to aspirin alone in achieving more live births in patients with positive aPL antibodies and RPL.

Key words. Heparin, Aspirin, Anti-phospholipid antibody syndrome, Live births, Obstetrical, Meta-analysis, Meta-regression, Egger’s regression.

Introduction

APS is characterized by clinical syndrome of vascular thrombosis and/or recurrent pregnancy losses (RPLs) associated with serological features of persistent elevation of the aPL antibodies [1]. Despite more than a decade of research, substantial evidence on the treatment to enhance successful pregnancy and live birth in mothers with APS is grossly lacking. While a number of guidelines, including a recent review, advocated the use of low-dose aspirin (LDA) and heparin in mothers with RPL and
positive aPL, these were mainly based on expert opinion rather than high-quality evidence from randomized controlled trials (RCTs) [2, 3]. In a recent guide for clinicians and researchers based on a questionnaire survey of experienced physicians and board members of a congress, the use of LDA and heparin was recommended during pregnancy as well as ovarian stimulation during *in vitro* fertilization for aPL-positive patients [4]. Although a Cochrane systemic review concluded that the combination of unfractionated heparin (UFH) and aspirin is more efficacious than aspirin alone in reducing RPLs or fetal wastage in APS, the analysis was based only on two RCTs and thus a sound conclusion could not be convincingly drawn [5]. The reasons for the lack of evidence are many. Besides the paucity of RCTs testing the treatment of APS-related pregnancy, other factors such as small sample size, difference in study design, heterogeneity in obstetric history and method of aPL profiling in the existing RCTs are operant.

Despite these facts, a few RCTs have been published since 1990s that tried to address the efficacy and safety of heparin/aspirin in the prevention of pregnancy losses and obstetrical complications. This prompts to perform an updated meta-analysis by combining the data of these trials to generate more insights into the current situation regarding evidence-based treatment of APS pregnancy. In brief, meta-analysis is a statistical procedure that combines results of eligible studies to generate a single estimate of the major effect with enhanced precision. It is regarded as a powerful tool for summarizing inconsistent results from different studies [6]. In the present meta-analysis, we aimed to assess whether the combination of heparin and aspirin is superior to aspirin alone in the prevention of pregnancy losses and obstetrical complications in patients with RPLs with positive aPLs.

**Materials and methods**

**Search strategy**

We performed a literature search using the relevant keywords of ‘antiphospholipid’, ‘pregnancy’, ‘heparin’, ‘aspirin’, ‘antiplatelet’, ‘lupus’ and ‘anticoagulants’ to identify RCTs published in English from different computerized databases: PubMed (1966–April 2009), EmBASE (1980–April 2009) and the Cochrane Centre Register of Controlled Trials (Issue 1, 2009). Bibliographies of the retrieved trials and review articles were also scanned and searched manually for relevant papers. We predetermined to contact the corresponding authors if essential information for this meta-analysis was lacking in the published articles.

**Criteria for selecting articles included in this meta-analysis**

Trials that randomly assigned patients to receive either the combination of heparin and aspirin or aspirin alone for the management of APS-related pregnancies were included. RCTs were included if they met the following criteria: (i) compare heparin and aspirin vs aspirin alone; and (ii) at least one of the following outcomes was reported: live births, pre-eclampsia, birth weight, prematurity, premature rupture of membranes (PROM) and fetal death.

Two investigators (A.M. and R.C.H.) independently assessed the relevance of the papers and papers with the following exclusion criteria were eliminated: (i) abstract not written in English; and (ii) not comparing the combination of heparin and aspirin and aspirin alone in preventing miscarriages/fetal losses and other unfavourable pregnancy parameters. Data were independently extracted into a standard electronic data extraction form. Any discrepancies were resolved by consensus. If consensus could not be reached, the principal investigator (A.M.) would make the final judgement for trial eligibility and data extraction. The statistician (M.W.-L.C.) carried out the statistical analyses and provided statistical advise of this meta-analysis.

**Outcome measures of meta-analysis**

The primary outcome was proportion of pregnancies resulting in live births. Secondary outcome measures included the proportion of mothers who had obstetrical complications, namely preterm labour, pre-eclampsia, PROM and Caesarean section. The mean birth weight of newborns in both groups was compared.

**Assessment of quality of trial**

The quality of each trial was assessed according to a standard scoring system proposed by Jadad et al. [7]. The assessment was based on: (i) whether the randomization method was appropriate; (ii) whether double blindness was mentioned in the trial and whether it was appropriately performed; and (iii) whether the number of patients and the reasons for withdrawal and dropouts were clearly stated. The score ranges from 0 to 5, with higher scores denoting better quality of trial.

**Statistical analysis**

The proportion of patients who gave live births was pooled for combined relative risk (RR). The RRs of the pre-defined secondary outcomes at the end of the study period were pooled. Effective sizes of both the primary and secondary outcomes were expressed as RRs and the corresponding 95% CI. Birth weights of newborns were pooled and expressed as the standard mean of difference (S.M.D.). All statistical pooling for effective sizes was performed using the inverse variance method. Additionally, the number needed to treat (NNT) one live birth was calculated by the reciprocal of the risk difference.

Heterogeneity was assessed by the Cochrane Q test. Since the number of trials of this meta-analysis is small, the Cochrane Q test for heterogeneity may yield a low statistical power [8, 9]. Furthermore, we assessed heterogeneity with $I^2$, which describes the percentage of total variation across studies caused by heterogeneity rather than chance. High values of $I^2$ suggest...
increased heterogeneity. Due to perceived considerable heterogeneity among different trials, we applied the random effects model with the method suggested by DerSimonian and Laird [10].

Meta-regression was performed to identify study-related factors that might predict the effective size [11]. These factors were age at randomization, history of early and late miscarriages and history of live births. Since the covariates chosen were not expected to explain all the heterogeneity of the trials, mixed-effects meta-regression was used in the consideration of the presence of ‘residual heterogeneity’ [11]. The regression coefficients and the associated s.e., the z-score and P-values were reported for the meta-regression analysis.

To assess the robustness of the effective sizes, sensitivity analyses were performed and they were divided into two parts. In the first part, trials using UFH and low molecular weight heparin (LMWH) were separately analysed and robustness of the outcomes was tested. In the second part, we evaluated the robustness of the pooled effective sizes by including study or studies with slightly different study characteristics but with the same objective.

Agreement between investigators

The inter-rater reliability agreement of the two investigators involved in literature search and inclusion (A.M. and R.C.H.) in terms of study inclusion or exclusion and quality of papers were assessed based on the Fleiss kappa (κ)-statistics [12].

Publication bias was assessed by Egger’s regression. The meta package implemented in R Development Core Team was used to conduct the random effects meta-analysis and create the forest plots [13]. MiMa was used to conduct the meta-regression [14]. The QUORUM statement for improving the quality and result of meta-analyses of RCTs was adhered where appropriate [15].

Results

Result of literature search

We initially identified 40 articles through database searches. In the first stage, we excluded clinical trials not apparently comparing the combination of aspirin and heparin and aspirin alone (n = 9), irrelevant trials (n = 15), non-RCTs (n = 2) and five reviews and/or meta-analyses (n = 5). In the second stage, when we focused on scrutinizing the nine RCTs, we eliminated two RCTs that compared the efficacy of high-dose heparin with aspirin plus low-dose heparin and one that compared aspirin with placebo. In the third stage, we further reviewed the remaining six RCTs, which compared the efficacy of the combination of heparin and aspirin and aspirin alone for eligibility. One study was excluded because prednisolone was used in patients in the aspirin group [16]. Another study, instead of being a truly randomized trial, assigned treatment alternatively [20]. Since dummy treatment was essentially absent in all the RCTs and they were all observational in nature after treatment assignment, we decided to include this study [20] in the meta-analysis. Thus, after the third stage of exclusion, the definitive analysis involved in this meta-analysis included five clinical trials published between 1996 and 2009 [16–21]. The result of the literature search is summarized in Fig. 1.

Among the 334 patients involved in these trials, 171 received heparin and aspirin, whereas 163 were assigned to take aspirin only. The mean age of the participants was 33.38 years. Eighty-seven (13.4%) of the participants recruited in these trials had history of recurrent early pregnancy and fetal losses, respectively, except for one study in which this information was not presented [17]. None of the participants had history of clinical vascular thrombosis.

For the quality of trials as assessed by the Jadad method [7], the mean (range) score was 1.6 (range 0–3). There are a few reasons why the quality of the trials involved in this meta-analysis is low. Double blindness was not possible in all these trials due to obvious ethical reasons related to research involving pregnant subjects. In addition, one study employed alternate assignments [19], whereas all, except one study [18], did not explicitly state how many and why patients dropped out from the trials [17, 19–21]. Table 1 summarizes the details of the RCTs included in this meta-analysis.
Agreement between investigators

The inter-rater reliability agreement of the two investigators (A.M. and R.C.H.) in terms of inclusion and exclusion of studies and quality of papers assessed by the Jadad’s method were 0.71 and 1.0, respectively, calculated based on the Fleiss’ $k$-statistic [12]. Such a level of agreement is considered to be both substantial and perfect [22].

Primary outcome

Summarizing the five RCTs, the overall live birth rates were 74.27% (127/171) and 55.83% (91/163) in patients who received the heparin/aspirin combination and aspirin alone, respectively (Table 2). Using the random effects model, patients who received heparin and aspirin had significantly higher live birth rate (RR 1.301; 95% CI 1.040, 1.629) than patients who received aspirin alone (Fig. 2). The NNT, combining heparin and aspirin to result in one live birth, is 5.6. No significant publication bias was noted by the Egger’s regression test ($P = 0.140$, two tailed).

Secondary outcomes

As far as pregnancy and obstetrical complications are concerned, patients on heparin and aspirin combination tended to have less pre-eclampsia (RR 0.471; 95% CI 0.096, 2.314) compared with patients on aspirin alone. There was also no significant difference in the risk for preterm labour (RR 1.027; 95% CI 0.399, 2.645) (Figs 3 and 4).

During the calculation of the S.M.D. of birth weights, the S.D.s of birth weight from one of the studies were missing [18]. Multiple imputations with 1000 imputations were used to estimate the S.M.D. and its S.E. [23]. There was no significant difference in birth weight between both groups (S.M.D. 0.084; 95% CI $-0.239$, $-0.408$) (Fig. 5). Egger’s regression test showed no significant publication bias in pooling effective sizes for pre-eclampsia ($P = 0.519$, two tailed), preterm labour ($P = 0.565$, two tailed) and birth weight ($P = 0.750$, two tailed). Effective size calculation for the proportion of PROM and that for

Table 1  Characteristics and quality of controlled trials comparing heparin and aspirin and aspirin alone in patients with obstetrical APS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Randomization</th>
<th>Inclusion criteria</th>
<th>Comparison</th>
<th>Age, mean, years</th>
<th>Previous miscarriages Early</th>
<th>Late</th>
<th>Previous live birth</th>
<th>Jadad score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuttet [20]</td>
<td>Alternate assignments</td>
<td>Three or more consecutive pregnancy losses and two separate positive aPL antibodies</td>
<td>UFH 10 000–20 000 U b.d. + aspirin 81 mg q.d. ($n = 25$) vs aspirin 81 mg q.d.</td>
<td>33.4</td>
<td>39/50</td>
<td>11/50</td>
<td>30/50</td>
<td>1</td>
</tr>
<tr>
<td>Rai et al. [18]</td>
<td>Computer-generated codes</td>
<td>Three or more consecutive pregnancy losses and two separate positive aPL antibodies</td>
<td>(n = 25) UFH 5000 U b.d. + aspirin 75 mg q.d. ($n = 45$) vs aspirin 75 mg q.d. ($n = 45$)</td>
<td>33.0</td>
<td>86/90</td>
<td>4/90</td>
<td>33/90</td>
<td>3</td>
</tr>
<tr>
<td>Farquharson et al. [17]</td>
<td>Computer-generated codes</td>
<td>Three or more consecutive pregnancy losses or two or more fetal deaths and two separate positive aPL antibodies</td>
<td>LMWH 5000 IU q.d. + aspirin 75 mg q.d. ($n = 51$) vs aspirin 75 mg q.d. ($n = 47$)</td>
<td>33.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
</tr>
<tr>
<td>Franklin and Kutteh [19]</td>
<td>ND</td>
<td>Two or more unexplained pregnancy losses and two separate positive aPL antibodies</td>
<td>UFH 5000–6000 IU b.d. + aspirin 81 mg q.d. ($n = 28$) vs aspirin 81 mg q.d. ($n = 26$)</td>
<td>33.3</td>
<td>75/79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45/79</td>
<td>0</td>
</tr>
<tr>
<td>Laskin et al. [21]</td>
<td>Randomized study numbers</td>
<td>Two or more unexplained pregnancy losses and two separate positive aPL antibodies</td>
<td>LMWH 5000 IU q.d. + aspirin 81 mg q.d. ($n = 22$) vs aspirin ($n = 20$)</td>
<td>34.2</td>
<td>66/88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22/88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30/88</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Included patients were with aPL antibodies other than aCL and anti-β2 glycoprotein I antibodies and LAC. <sup>b</sup>Included patients were with other causes of thrombophilia other than APS. ND: no data; b.d. twice daily; q.d.: once daily.
patients undergoing Caesarean section was not done due to insufficient data.

Except mild heterogeneity, which was noted in pooling effective size for live birth rate ($I^2 = 46.4\%$), no substantial heterogeneity was noted in the rest of the analyses (Figs 2–5).

Sensitivity analysis
The sensitivity analysis comprised two parts. In the first part, studies that used LMWH and UFH were analysed separately. The results remained robust except that there was just a trend of higher live birth rate in patients receiving LMWH. Secondly, we re-analysed the data by adding the study of Cowchock et al. [16] in which prednisolone was added in the aspirin group. The results remained robust except that the pre-eclampsia rate was significantly lower, whereas the preterm labour rate tended to be lower in patients who received heparin and aspirin compared with those who took only aspirin (Table 3).

Meta-regression
Mixed-effects univariate meta-regression using age at randomization, previous history of live births and early and late miscarriages failed to predict the RR of live birth (Table 4).

Discussion
The current meta-analysis demonstrated the superiority of the heparin and aspirin combination with aspirin alone in
terms of higher live birth rates in patients with positive aPLs who experienced RPL. Patients who received the aspirin and heparin combination were 1.3 times more likely to result in live birth than those who took aspirin alone during pregnancy, with an NNT of 5.6 for achieving one live birth. No statistically notable publication bias was observed in all the analyses.

We noted two interesting points in the sensitivity analyses. First, there was a significant reduction of 72% in pre-eclampsia in patients who received heparin and aspirin when the study of Cowchock et al. [16] was included into the sensitivity test. Secondly, the preterm rate was much lower, not reaching statistical significance, in patients who had a combination of heparin and aspirin when Cowchock et al.’s study was included (Table 3). In this study [16], prednisolone, the drug that has been out of favour and actually postulated to be harmful in the treatment of obstetrical APS [3], was given to the aspirin-only group. On closer examination of Cowchock et al.’s study, the rates of pre-eclampsia and preterm births were much higher in the aspirin group compared with other studies (Table 2). Thus, it is not surprising that when this study was included in the meta-analysis, the risk ratios of pre-eclampsia and preterm births would be lower as prednisolone might exaggerate the risk of these two complications in the aspirin group. Such findings may also serve to further illustrate the potential disadvantage of administering glucocorticoid in patients with APS with regard to the complications of pre-eclampsia and preterm births.

### Table 4 Meta-regression analysis of potential factors contributing to heterogeneity of live birth

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Regression coefficient (S.E.)</th>
<th>z-score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at randomization</td>
<td>−0.1719 (0.210)</td>
<td>−0.817</td>
<td>0.414</td>
</tr>
<tr>
<td>Previous live births</td>
<td>1.1720 (0.951)</td>
<td>1.232</td>
<td>0.218</td>
</tr>
<tr>
<td>Previous early miscarriages</td>
<td>1.4215 (1.087)</td>
<td>1.308</td>
<td>0.191</td>
</tr>
<tr>
<td>Previous late miscarriages</td>
<td>−1.4194 (1.086)</td>
<td>−1.307</td>
<td>0.191</td>
</tr>
</tbody>
</table>

### Table 3 Results of sensitivity analyses

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>RR (five studies)</th>
<th>RR (studies involving UFH)</th>
<th>RR (studies involving LMWH)</th>
<th>RR (including Cowchock et al.’s study [16])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth</td>
<td>1.301 (1.040, 1.629)</td>
<td>1.636 (1.262, 2.119)</td>
<td>1.067 (0.883, 1.290)</td>
<td>1.252 (1.036, 1.512)</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>0.471 (0.096, 2.314)</td>
<td>0.471 (0.096, 2.314)</td>
<td>Not estimable</td>
<td>0.283 (0.082, 0.982)</td>
</tr>
<tr>
<td>Preterm</td>
<td>1.027 (0.399, 2.645)</td>
<td>1.599 (0.501, 5.098)</td>
<td>Not estimable</td>
<td>0.626 (0.217, 1.808)</td>
</tr>
<tr>
<td>Birth weight</td>
<td>0.084 (−0.239, −0.408)</td>
<td>0.225 (−0.178, −0.628)</td>
<td>Not estimable</td>
<td>0.084 (−0.239, −0.408)</td>
</tr>
</tbody>
</table>

The 95% CI values are given within parentheses. *Either no study or only one study is involved.*
The sensitivity analyses also demonstrated that the results were generally robust. Although when trials involving LMWH were pooled, the RR of live births was no longer significant in the combination group; it was only based on two trials. Although two recent RCTs comparing the combination of either LMWH or UFH with aspirin revealed no significant difference in fetal outcomes and maternal complications in either form of heparin, both RCTs were grossly underpowered to detect a significant difference [24, 25]. With the increasing use of LMWH due to its potential advantages over UFH such as lower risks of haemorrhage, thrombocytopenia, bone loss and easier administration, more trials involving the use of LMWH will shed more light on whether both forms of heparin are equally efficacious in preventing pregnancy loss and complications in patients with obstetric APS.

The exact mechanism of how heparin reduces pregnancy loss is still not fully understood. Apart from being able to prevent thrombosis and subsequent placental infarcts by its anti-coagulant effect, heparin was also shown to bind to aPLs and render them biologically inactive [26]. More recently, it was shown that complement system activation, especially those involving C3 and C5, is an essential mechanism for aPL-induced fetal loss. Since activation of C3 and C5 promotes pro-coagulant effect of aPLs, heparin, apart from being an anti-coagulant, can increase the chance of successful implantation of embryo by inhibiting the pro-coagulant effect as a result of C3 and C5 activation [27]. Besides protection against pregnancy loss, heparin was also demonstrated to reduce obstetrical complications such as pre-eclampsia and low birth weight in a recent small clinical trial [28].

There are several limitations to this study, which mainly stem intrinsically from meta-analysis and quality of the clinical trials included. First, with the relatively small number of trials involved in this meta-analysis, the result of this study is subjected to random error. Secondly, sample size of the trials may also affect the fidelity of the pooled RRs. We calculated a posteriori the number of patients required to demonstrate the risk observed, with a statistical power of 80% and an α-level of 5%. We found that 96 patients would be necessary in both the treatment and placebo groups. Although no individual study involved in this meta-analysis was powered enough to detect the difference, the current meta-analysis may offer sufficient power to detect the true difference in live birth rates between heparin and aspirin and aspirin alone. Thirdly, although no statistically significant publication bias was noted by using Egger’s regression, there is no perfect method to assess and quantify publication bias and hence, such potential bias may distort and confound the interpretation of the results of this meta-analysis [29]. Fourthly, due to the impossibility of double blindness, alternate assignment of treatment in one study [20] and lack of explicit statement of how many and why patients withdrew in the majority of the trials [17, 19–21], the quality of all the trials involved in this meta-analysis was low. The quality of the suboptimal study may mitigate the fidelity of the effective size when these trials were combined. Finally, with the missing information, a number of obstetrical complications such as the risks of PROM and Caesarean section could not be analysed. Since the majority of the studies were published some time ago, it was difficult for the authors to retrieve the information. The presence of these limitations means that extra caution is necessary when we interpret the results and conclusion of this meta-analysis.

Performing RCTs in patients with pregnancy has long been a great challenge, in terms of willingness to participate, difficulty in recruitment and various ethical issues with regard to placebo use. For example, in Laskin et al.’s study [21], less than one-tenth of the participants screened were eventually eligible for the study. This even poses a specific challenge if only patients with APS are studied because it constituted only ~40–50% of mothers with history of RPL. Furthermore, patients with SLE or other primary autoimmune diseases were excluded in the RCTs involved in this meta-analysis. Therefore, the results cannot be truly extrapolated in patients with secondary APS.

Before a truly randomized trial with a larger sample size (more than 96 per groups) can be successfully performed and published, the current meta-analysis offers the most updated evidence showing that the combination of heparin and aspirin is superior to aspirin alone in increasing successful pregnancy in terms of higher live birth rates in mothers with RPL and positive aPLs.

Conclusions

Combination of heparin and aspirin appears superior to aspirin alone in patients with RPL and positive aPLs in terms of more live births. The NNT is 5.6 for such a combination to result in one live birth.

Rheumatology key messages

- The heparin and aspirin combination is superior to aspirin alone in enhancing live birth in obstetric APS.
- The NNT of aspirin and heparin to achieve one live birth is 5.6.

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References